ZOOLOGY.—*The development of the Trichostrongyle,* Nippostrongylus muris, *in rats following ingestion of larvae.*[1] BENJAMIN SCHWARTZ and JOSEPH E. ALICATA, Bureau of Animal Industry.

In the course of investigations on the life history of the dog hookworm, *Ancylostoma caninum,* the view has been advanced by certain investigators that the larvae of this helminth are capable of developing in the intestine of dogs without migration to the lungs. These parasites usually migrate to the lungs when introduced through the mouth into the bodies of small laboratory animals. The direct development of *A. caninum* in dogs was first noted by Yokogawa (7) and was later confirmed by Scott (5). Fuelleborn (2) reported the same findings as a result of experimental infections with another species of dog hookworm, *Uncinaria stenocephala,* and he observed, moreover, that only a small percentage of the larvae of *U. stenocephala* took the roundabout journey to the lungs even in abnormal hosts. While these conclusions are not accepted by all investigators, having been challenged especially by Nakajima (4) and Nagoya (3), the data which have been published in support of what appears to be a rather unorthodox view, seem to be sound and convincing.

During the past few years the writers have been engaged in experimental investigations on the course of infection of rats with *Nippostrongylus muris,* a trichostrongyle nematode of wild rats, readily transmissible to white rats. Several experiments were undertaken with a view to determining whether the migration of the larvae of this species to the lungs is an essential part of the developmental process within the host, or whether the occurrence of the larvae in the lungs is merely the result of the worms being accidentally arrested in the lungs while en route from the skin to the intestine.

Yokogawa (6), who first determined the life history of *N. muris,* noted that white rats could be infected with this worm through the mouth as well as percutaneously; the resultant infestation following the introduction of larvae into the mouth was very slight, and in some cases no infestation could be produced. According to this investigator the infestation resulting from a percutaneous infection was always proportionate to the number of larvae applied to the skin. Africa (1) noted that the intensity of experimental infections of rats by mouth with *N. muris,* as judged by the number of eggs eliminated with the feces of infested animals, was very low as compared to the number of eggs eliminated from percutaneously infected hosts when the rats in both groups were given equal numbers of larvae.

[1] Received May 21, 1934.

While the available evidence indicated that the skin was the more normal portal of entry of *N. muris* into the body of its host, and that the entry of the larvae through the mouth produced but slight infestations or no infestations, no data were available to account for these results. The data obtained by the writers not only have cleared up this point in the life history of *N. muris* but also have a bearing on the question of the biological significance of the migration of the larvae of this species from the intestine to the lungs.

Infective larvae from cultures of rat feces mixed with animal charcoal were introduced into the mouths of 10 rats in a few drops of water with the aid of a small pipette. Rats 1, 5, 9 and 10 received 2,000 larvae each; the remaining rats received 1,000 larvae each. The experimental rats were killed with ether, and were examined post mortem for evidence of worm infestation. The lungs were chopped into small pieces with a pair of fine scissors and put through the Baermann apparatus; this was followed by an examination of each fragment of lung tissue in press preparation. The stomach, small intestine, and large intestine were examined separately with the aid of the Baermann apparatus. Examinations for larvae were also made of the hearts' blood and, in some cases, of scrapings of the intestinal mucosa, as well as of the liver and intestinal lymph glands; the latter two tissues were examined in press preparation and with the Baermann apparatus. In a number of instances the feces of the experimental rats were examined for larvae.

PROTOCOLS

Rat 1 (killed 3 hours after feeding of larvae)—15 infective larvae in small intestine, one of which was obtained by scraping the mucosa; 95 infective larvae in large intestine; some of the larvae from both locations sluggish, giving but a feeble response to tactile stimulation with the dissecting needle; other larvae normally active.

Rat 2 (killed 3 hours after feeding of larvae)—9 infective larvae in stomach; 25 infective larvae in small intestine; 32 infective larvae in large intestine. All larvae alive.

Rat 3 (killed 5 hours after feeding of larvae)—10 live larvae in large intestine.

Rat 4 (killed 18 hours after feeding of larvae)—2 dead larvae in large intestine and 23 dead larvae in feces.

Rat 5 (killed 24 hours after feeding of larvae)—9 live third-stage larvae in lungs.

Rat 6 (killed 30 hours after feeding of larvae)—9 live third-stage larvae in lungs; 1 dead larva in large intestine.

Rat 7 (killed 35 hours after feeding of larvae)—10 live third-stage larvae in lungs; 2 dead larvae in large intestine.

Rat 8 (killed 42 hours after feeding of larvae)—5 live third-stage larvae in lungs.

Rat 9 (killed 48 hours after feeding of larvae)—5 live fourth-stage larvae in small intestine.

Rat 10 (killed 72 hours after feeding of larvae)—2 live larvae in lungs; 44 live fourth-stage larvae in small intestine.

DISCUSSION

It is evident from these data that following the ingestion of infective larvae of *N. muris* by white rats, the worms passed into the stomach, where they were found 3 hours after ingestion. From the stomach the larvae passed into the small intestine in which location some of them became sluggish. Many larvae passed down into the large intestine where they were found still alive 5 hours after ingestion. The larvae died in the large intestine without undergoing any development and passed out with the feces (rat 4). A small number of larvae apparently bored into the wall of the small intestine, as evidenced by the discovery of 1 larva in the scrapings of the intestinal epithelium of rat 1, and subsequently got to the lungs.

In the series of experiments described in this paper, larvae were not found in the lungs from 3 to 18 hours following their ingestion; larvae were recovered from the lungs of experimentally infected rats from 24 to 72 hours after experimental feeding. While larvae, showing no development beyond that of the infective stage, were found in rats 3 hours after feeding, they were absent from the small intestine between 5 and 42 hours after feeding, reappearing again as fourth-stage larvae from 48 to 72 hours after feeding. These observations are in agreement with those of Yokogawa (6) who noted that following percutaneous infection, *Nippostrongylus* larvae appeared in the lungs in from 14 to 20 hours, remained in this location until 50 to 65 hours, and occasionally as late as 72 hours, after having been placed on the skin.

The development and migration of *N. muris* in white rats, following ingestion of infective larvae, followed the same course as observed by Yokogawa (6) subsequent to percutaneous infection. The larvae migrated to the lungs, where they continued their development to the

fourth stage[2] and finally returned to the small intestine. The larvae which failed to penetrate the intestinal mucosa following ingestion, and apparently the vast majority of larvae introduced into the mouth did fail, passed into the large intestine where they died and were subsequently eliminated from the body. No evidence was found of the development of larvae in the intestine without migration to the lungs. Apparently the series of developmental changes in *N. muris* which takes place in the lungs of the host and which culminates in an ecdysis, did not occur as long as the worms remained in the intestine. The migration of the larvae to the lungs appears to be an essential step in the developmental history of these parasites, the worms not being adapted to survival in the small intestine until the completion of the second ecdysis in the lungs; the first ecdysis takes place outside of the host, the larva attaining the infective stage following the first molt, and the final ecdysis takes place in the intestine.

SUMMARY

When introduced into the mouths of white rats, *Nippostrongylus muris* passed into the stomach, some larvae surviving in the stomach for 3 hours, and thence into the small intestine. A relatively small percentage of larvae evidently penetrated the intestinal mucosa and reached the lungs. Other larvae passed into the large intestine where they died and were expelled with the feces.

The larvae which migrated from the intestine to the lungs developed in the normal way; larvae were found in the lungs of white rats from 24 to 72 hours after experimental feeding.

Live larvae showing no development beyond that of the infective stage were found in the intestine of white rats up to 5 hours after experimental feeding; no larvae were found in the small intestine from 18 to 42 hours after experimental feeding, though in three out of four rats involved larvae were present in the lungs during these periods. Two rats killed 48 and 72 hours, respectively, after experimental feeding, contained fourth-stage larvae in the intestine.

The data presented in this paper indicate that the larvae did not develop in the small intestine to the fourth stage, but that the time of the appearance of fourth-stage larvae in the small intestine, coupled with the finding of larvae in the lungs, warrants the conclusion that

[2] According to Yokogawa (6) *N. muris* molts only three times, namely, once as a free-living larva, once in the lungs and once in the intestine. The writers have followed Yokogawa in regarding the development of the larvae in the lungs as involving two stages, one molt being suppressed.

the third-stage larvae developed in the lungs and returned to the intestine as fourth-stage larvae.

The migration of *N. muris* to the lungs appears to be an essential part of their life history, irrespective of their path of entry into the body of the host.

It may be concluded that *N. muris* passes through three distinct developmental phases in the course of its life cycle, namely: (1) The free-living stage, involving one ecdysis, following which the worms emerge as second-stage infective larvae; (2) the pulmonary parasitic stage, in the course of which the larvae develop to the third stage and finally to the fourth stage, the latter stage preceded by an ecdysis; (3) the intestinal parasitic stage in the course of which the larvae grow to the fifth or final stage, preceded by a third ecdysis, followed subsequently by the development of the worms to fertile maturity.

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This paper was read at a meeting of the Third Pan-Pacific Science Congress held in Tokyo in 1926. The date of publication is uncertain.

ZOOLOGY.—Sphaeropomatus miamiensis, *a new genus and species of Serpulid polychaete.*[1] Aaron L. Treadwell. (Communicated by Mary J. Rathbun.)

In May 1933, Captain John W. Mills presented to the United States National Museum an unusually large and perfect specimen of the fresh-water shrimp *Macrobrachium jamaicense* from the Miami River,

[1] Published by permission of the Secretary of the Smithsonian Institution. Received March 12, 1934.